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Effects of atrazine on soil microarthropods in experimental maize fields

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1. Introduction

Herbicides can influence population densities of soil fauna both directly by acting on their vital functions and indirectly by acting on their habitat (EIJSSACKERS & VAN DER DRIFT 1976). The more persistent the herbicide, the more evident will be the effects (EDWARDS 1969). Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) belongs to the triazine class of herbicides and is used for selective weedkilling in maize and sorghum crops.

Chemical studies show a rapid fall in herbicidal activity after a short period in the soil (WALKER 1978; BASILE 1981; CAVAZZA *et al.* 1982).

The literature presents numerous papers on the effects of herbicides on arthropods and other animal groups (for review see EDWARDS & THOMPSON 1973; EIJSACKERS & VAN DER DRIFT 1976; EDWARDS & STAFFORD 1979; MADGE 1981), nevertheless, little data are available on the effects of atrazine on microarthropods, both in laboratory and field studies.

SUBAGJA & SNIDER (1981) reared the Collembola *Folsomia candida* and *Tullbergia granulata* on brewer's yeast treated with particularly high doses of atrazine (5,000 ppm) and found that fecundity was reduced and instar duration lengthened.

Effects of this herbicide on Lumbricidae, Collembola and Elateridae have been reported to persist even 14 months after treatment with doses of 9 kg ha⁻¹ (Fox 1964).

A synergic effect of atrazine with some insecticides has been found on the larvae of Diptera (LIANG & LICHTENSTEIN 1974; LICHTENSTEIN *et al.* 1975).

POROVICI *et al.* (1977) studied the effects of atrazine at doses of 5 and 8 kg ha⁻¹ in a maize cultivated field; soil samples showed decreases in numbers of Protozoa, Enchytraeidae, Acarina, Collembola and adult Pterygota populations, one and four months after herbicide treatment. According to the authors, a direct relationship existed between herbicide doses and reductions in the number of animals. Within 4 months, a partial recovery of populations was found. No effects were found on nematodes, a finding also reported by FREY (1976).

Repeated herbicide treatments in alternate years with doses of up to 6 kg ha⁻¹ did not produce any effects on microarthropod populations (SABATINI *et al.* 1979). Coordinated research in the same experimental field provided various data on the effects of atrazine. On the microbial components of the soil, FAVILLI *et al.* (1981) found a reduction in cyanobacters in the treated plots after one year, while diatoms were affected by the herbicide for only a few months. Number of green algae were reduced at doses greater than 3 kg ha⁻¹ but only in the period immediately following herbicide application: after 6 months their numbers returned to that in untreated soil.

2. Description of fields

Two experimental fields cultivated with maize were studied: a) Field No. 9 of the "Azienda Agricola di Cadriano" of the University of Bologna, which had been treated with 2 kg ha⁻¹ of atrazine two years prior to the study. This field lies adjacent to Field No. 8 where SABATINI *et al.* (1979) performed their research.

b) C.N.R. field (originally part of the ex-“Consorzio Canapa”) of Vitulazio (Caserta), which had never been previously treated with atrazine.

The Cadriano field is characterized thus: 55 % sand, 25 % silt, 20 % clay, 1.8 C, 0.15 N, 24 mEq/100 g C.S.C.; pH 6.7 (CATIZONE *et al.* 1983).

The Vitulazio field composition for the top 50 cm was: 53 % sand, 20 % silt, 26 % clay, 0.81 C, 0.13 N, 13.4 mEq/100 g C.S.C.; pH 8.0 (BUONDONNO 1982).

Other types of coordinated research had been done on the same fields. At Cadriano CERVELLI & CIARDI (1981) studied urease activity, concentrations of ammonium ions, nitrates, nitrites, total nitrogen, and pH of the soil, and found that atrazine affected the nitrogen cycle. Atrazine reduced urease activity and increased nitrate concentrations subsequent to increased ammonification.

The superficial soil layers of the Cadriano field showed residual atrazine five months after the treatment, and atrazine metabolites were found in concentrations of 0.11 ppm from an initial dose of 3.64 ppm (4 kg ha⁻¹) and were below the limit of sensibility of the method (0.05 ppm) from an initial dose of 1.82 ppm (2 kg ha⁻¹). In the Vitulazio field atrazine residues were 0.2 ppm and 0.1 ppm after 4 months, from initial concentrations of 4 and 2 kg ha⁻¹ respectively (TAFURI 1981).

3. Material and methods

Soil samples were taken 7 days (Sample Period I, in May) and 30 days (Sample Period II, in June) after herbicide application from plots in the two fields treated in May 1979 with doses of 2 kg ha⁻¹ (recommended agricultural dose) and 6 kg ha⁻¹ and from untreated plots (0 kg ha⁻¹).

Control plots were weeded by hand and kept weed free for the duration of the experiment in order to provide the same vegetational conditions in both the atrazine-treated and atrazine-free plots.

Undisturbed soil cores (10 cm × 10 cm × 3 cm) were cut from the trench walls with small shovels. They were taken at two different depths one on the top of the other (0–10 cm and 10–20 cm), with 8 replicates from each of the three treatments; samples were sealed in small polyethylene bags and transported to the laboratory.

Microarthropods were extracted using a modified Macfadyen type extractor (SABATINI *et al.* 1979) followed by mechanical extraction on the same soil samples by the flotation method according to HALE (1964).

Preliminary observations have already been reported (BERTOLANI *et al.* 1981).

Specific taxonomic diagnoses were made only for Colembola, and the list of species in the two fields is given in Table 1.

The statistical analysis of the data was based on a split-plot design. Each treatment (control, 2 kg ha⁻¹ and 6 kg ha⁻¹) was considered as a “plot” and the superficial (0–10 cm) and deep (10–20 cm) layers as “sub-plots”; the eight samples taken from each plot were replicates.

The distinct populations of the 0–10 cm and 10–20 cm layers and the constancy of the latter populations in all the plots considerably reduced the sensitivity of the split-plot analysis when comparing values from the same depth. Therefore we evaluated the least significant difference of the mean (L.S.D.) when comparing samples coming from the same layers (SCOSSIROLI & PALENZONA 1971).

4. Results and discussion

In the Cadriano and Vitulazio fields, both in the control and in the atrazine-treated plots, the microarthropods had a normal overall distribution; in particular normal distribution had also Acarina and Collembola. *Mesaphorura krausbaueri*, *Willemia buddenbroeki* and *Isotomiella minor* (Collembola), in a sample (out of 96) different for each species, had a number of animals larger than $\mu + 3\sigma$ of the specific set of samples, suggesting a non-normal distribution. The data were omitted but μ and σ were computed considering the data within the samples.

The two fields studied had qualitative and quantitative differences in microarthropod population (Tables 1 & 2). In both fields Acarina and Collembola were the most numerous. There was also a distinct distribution of animals between the 0–10 and 10–20 cm layers: Collembola were more numerous in the superficial layer at Vitulazio (Table 3) but in the deep layer at Cadriano (Table 4). Myriapoda were essentially Pauropoda and Symphyla (in the Cadriano samples they were 146 and 53 respectively on a total of 204 myriapods; in the Vitulazio samples 56 and 14 on a total of 74). Diplura have been found at Vitulazio but were lacking at Cadriano. Qualitative differences were found within the Collembola group: Neanuridae and Hypogastruridae were lacking at Cadriano. A greater number of Collembola species was found at Vitulazio: among these *Schaefferia scossirolii* (new to

Table 1. List of Collembola species found in the two experimental fields

Vitulazio	Cadriano
Arthropleona	
Neanuridae	
<i>Friesea truncata</i> CASSAGNAU, 1958	
<i>Friesea</i> cfr. <i>mauriesi</i>	
Hypogastruridae	
<i>Hypogastrura vernalis</i> (CARL, 1901)	
<i>Hypogastrura</i> sp.	
<i>Schaefferia scossirolii</i> DALLAI & SABATINI, 1981	
<i>Willemia buddenbrocki</i> HUETHER, 1959	
Onychiuridae	
<i>Mesaphorura krausbaueri</i> BOERNER, 1901	<i>Mesaphorura krausbaueri</i> BOERNER, 1901
<i>Neotullbergia ramicuspis</i> (GISIN, 1953)	
<i>Onychiurus armatus</i> (TULLBERG, 1869)	
<i>Onychiurus</i> sp.	
Isotomidae	
<i>Isotomiella minor</i> (SCHAEFFER, 1896)	<i>Isotomiella minor</i> (SCHAEFFER, 1896)
<i>Cryptopygus ponticus</i> (STACH, 1947)	<i>Cryptopygus thermophilus</i> (AXELSON, 1900)
<i>Isotoma</i> sp.	<i>Isotoma</i> sp.
<i>Folsomides parvulus</i> STACH, 1922	<i>Proisoloma minuta</i> (TULLBERG, 1871)
<i>Isotomodes sexsetosus provincialis</i> POINSOT, 1966	
Entomobryidae	
<i>Entomobrya nivalis</i> L.	<i>Entomobrya multifasciata</i> (TULLBERG, 1871)
<i>Heteromurus nitidus</i> (TEMPLETON, 1835)	<i>Heteromurus nitidus</i> (TEMPLETON, 1835)
<i>Pseudosinella</i> sp.	<i>Pseudosinella</i> sp.
<i>Pseudosinella imparipunctata</i> GISIN, 1953	<i>Pseudosinella albida</i> STACH, 1930
<i>Pseudosinella octopunctata</i> BOERNER, 1903	
<i>Cyphoderus albinus</i> NICOLET, 1841	
<i>Lepydoctus violaceus</i> LUBBOCK, 1873	
<i>Seira ferrarii</i> PARONA, 1888	
<i>Orchesella</i> sp.	
Symphypleona	
Sminthuridae	
<i>Megalothorax minimus</i> WILLEM, 1900	<i>Megalothorax minimus</i> WILLEM, 1900
<i>Sminthurides schoetti</i> AXELSON, 1903	

science — DALLAI & SABATINI 1981) and *Willemia buddenbrocki* (new to Italy). The following species were found only in limited numbers in the control or in the 2 kg ha⁻¹ treated plot of Vitulazio and absent from the 6 kg ha⁻¹ treated plot: *Hypogastrura vernalis*, *Friesea truncata*, *Megalothorax minimus*, *Pseudosinella octopunctata*, *P. imparipunctata*, *Seira ferrarii*, *Cyphoderus albinus*, *Heteromurus nitidus*. Only one species, *Isotomodes sexsetosus provincialis*, was found in the atrazine treated plots (6 specimens in 3 samples of the 0–10 cm layer and 46 specimens in 8 samples of the 10–20 cm layer) but not in the controls.

The main results of our study on microarthropod populations were the overall nil effect of atrazine in the Cadriano field and decrease in population densities at Vitulazio. Tables 2–4 summarize the results of statistical analysis applied to the data from Vitulazio and from Cadriano.

Animals living at a depth of 10 cm or more, both in the Vitulazio and Cadriano fields, were not affected by atrazine: this may be attributed to poor vertical diffusion of the herbicide (CAVAZZA *et al.* 1982).

Following herbicide treatments, at Vitulazio (Table 3) a decrease in the 0–10 cm layer populations of Acarina and Collembola was seen while the deeper populations did not generally show any changes. Consequently, the treated plots did not have significant differences in numbers between the 0–10 cm and 10–20 cm layers. The only exception was the

Table 2. Number of animals recovered, abundance and frequency of microarthropod populations under the

	kg	ha ⁻¹	Acarina				Myriapoda				Collembola	
			N	Nm ⁻²	σm ⁻²	%	N	Nm ⁻²	σm ⁻²	%	N	Nm ⁻¹
Vitulazio	I	0	425	17722	1146	46.9	34	1418	271	3.8	409	17055
		2	391	16305	1900	52.3	18	751	159	2.4	318	13260
		6	220	9174	1104	43.7	4	167	—	0.8	247	10300
	II	0	249	10383	353	40.7	10	417	43	1.6	316	13177
		2	409	17055	1391	58.3	6	250	—	0.8	238	9925
		6	182	7589	412	53.5	2	83	—	0.6	112	4670
Cadriano	I	0	186	7756	547	50.5	26	1084	172	7.1	141	5880
		2	273	11384	894	65.6	29	1209	127	6.8	111	4629
		6	329	13719	1041	66.2	40	1668	277	8.0	127	5296
	II	0	228	9508	663	40.0	33	1376	467	5.8	308	12844
		2	257	10717	684	53.0	32	1334	141	6.6	196	8173
		6	275	11467	854	45.4	44	1835	202	7.3	284	11843

Note: I = 7 days after treatment; II = 30 days after treatment; N = Number of animals recovered in all
 % = frequency per plot; 0 kg ha⁻¹ = control hand-weeded; 2 kg ha⁻¹ = atrazine in agricultural recom-

Table 3. Sample mean and least significant difference for the confidence level 95% (LSD) of the animal populations in the Vitulazio field in 0—10 cm and 10—20 cm layers under the influence of atrazine treatments.

Sample period		I (May)				II (June)			
Treatment dose (kg ha ⁻¹)		0	2	6		0	2	6	
Taxon	Layer	L.S.D.				L.S.D.			
Acarina	0—10 cm	36.1	19.7	8.7	15.3	24.6	32.2	15.6	14.9
	10—20 cm	8.5	12.5	9.7	23.5	6.5	12.6	7.1	6.7
Collembola	0—10 cm	36.7	18.7	11.5	17.0	26.5	12.2	5.9	10.9
	10—20 cm	14.4	21.0	19.4	14.0	8.6	8.6	8.1	8.1
Isotomidae	0—10 cm	6.5	2.5	1.0	3.7	5.5	1.9	3.1	4.4
	10—20 cm	1.9	2.0	1.2	2.5	0.5	2.5	1.6	3.9
Hypogastruridae	0—10 cm	17.9	8.9	5.5	10.0	9.9	4.0	0.9	7.9
	10—20 cm	5.0	6.1	10.4	7.0	6.2	2.9	5.0	7.5
Onychiuridae	0—10 cm	5.9	4.2	1.7	3.3	3.2	1.1	0.5	2.6
	10—20 cm	5.9	12.4	4.7	6.8	3.0	2.1	0.9	3.1
<i>Isotomiella minor</i>	0—10 cm	3.9	0.7*	0.4	1.7	3.4	1.6	0.4	2.6
	10—20 cm	0.9	0.6	0.4	0.8	0.4	2.4	0.6	2.9
<i>Willemia buddenbrocki</i>	0—10 cm	8.6	6.5	5.5*	8.6	9.6	2.0	0.7	7.7
	10—20 cm	4.6	6.1	10.4	7.0	5.9	2.6	5.0	7.5
<i>Mesaphorura krausbaueri</i>	0—10 cm	4.1	3.7	1.2	2.7	2.5	0.2	0.5	2.5
	10—20 cm	4.7	11.0	4.1	6.0	2.5*	1.6	0.9	2.9

* One datum, larger than $\mu + 3 \sigma$, was omitted (see: results and discussion).
 Note: The vertical bar indicates a significant difference ($P > 0.05$) between the two layers.

Onychiuridae (and their most abundant species, *M. krausbaueri*): seven days after treatment with 2 kg ha⁻¹ these were significantly more abundant in the 10—20 cm layer but did not present any significant variation in the 0—10 cm layer.
 The mean numbers of the Collembola Isotomidae, Hypogastruridae and Onychiuridae were significantly different between control and 6 kg ha⁻¹ atrazine treated plot ($P > 0.01$ for Isotomidae); in contrast the number of Hypogastruridae and Onychiuridae did not differ between the control and 2 kg ha⁻¹ treated plot. The mean numbers of *I. minor* was different in control and treated plots ($P > 0.01$), that of *M. krausbaueri* was different only

influence of atrazine treatments

		Diplura				Pterygota				total microarthropods		
σm^{-2}	%	N	Nm^{-2}	σm^{-2}	%	N	Nm^{-2}	σm^{-2}	%	N	Nm^{-2}	σm^{-2}
763	45.2	7	292	61	0.8	29	1209	124	3.2	904	37780	1760
898	42.5	1	42	—	0.1	20	834	116	2.7	748	31191	2099
1122	49.1	1	42	—	0.2	31	1293	147	6.2	503	20975	1695
1367	51.7	5	208	—	0.8	30	1251	109	4.9	610	25479	1486
1656	33.9	13	542	86	1.8	35	1460	165	5.0	701	29273	2836
243	32.9	0	—	—	—	44	1835	156	12.9	340	14428	542
181	38.3	0	—	—	—	15	625	145	4.1	368	15345	805
358	26.7	0	—	—	—	3	125	—	0.7	416	17347	1208
197	25.5	0	—	—	—	1	42	—	0.02	497	20725	1983
760	54.0	0	—	—	—	1	42	—	0.2	570	23770	1438
593	40.4	0	—	—	—	0	—	—	—	485	20225	1027
912	46.9	0	—	—	—	2	83	—	0.3	605	25228	1866

samples; Nm^{-2} = animals in 1 square metre $\times$ 20 cm deep; σm^{-2} = mean standard deviation per sq.m.; mended dose; 6 kg ha⁻¹ = 6 kg ha⁻¹ atrazine.

Table 4. Sample mean and least significant difference for the confidence level 95% (LSD) of Acarina and Collembola populations in the Cadriano field in 0–10 cm and 10–20 cm layers under the influence of atrazine treatments

Sample period		I (May)			II (June)			
Treatment dose (kg ha ⁻¹)		0	2	6	0	2	6	
Taxon	Layer	L.S.D.			L.S.D.			
Acarina	0–10 cm	13.4	18.9	18.5	9.5	20.4	22.1	19.9 12.9
	10–20 cm	9.9	12.9	14.7	7.9	8.1	10.0	14.5 8.5
Collembola	0–10 cm	6.5	5.7	3.7	3.9	12.2	8.2	12.7 9.4
	10–20 cm	11.1	8.1	12.1	8.3	25.7	16.2	22.7 16.0

Note: The vertical bar indicates a significant difference ($P > 0.05$) between the two layers.

between control and 6 kg ha⁻¹ plot; *W. buddenbrocki* had no differences between control and treated plots.

Thirty days after treatment, samples showed no significant differences for Acarina and significant differences for Collembola ($P > 0.01$ between control and treatment with 6 kg ha⁻¹). At the higher doses, significant differences between control and treated plot were found for Onychiuridae and Hypogastruridae but not for Isotomidae: *W. buddenbrocki* and *I. minor* were significantly decreased in the 6 kg ha⁻¹ treated plot with respect to control. Thus at Vitulazio atrazine treatment had group-specific effects on the animal populations: e.g. Acarina, Isotomidae and *M. krausbaueri* show an immediate though transitory fall in number after herbicide application while effects on other Collembola were much longer.

Neither of the statistical methods applied showed significant differences between the population densities in treated and untreated Cadriano plots (Table 4), further supporting the results found in a study in an adjacent field treated for several years with the same herbicide (SABATINI *et al.* 1979). Therefore it seemed that the reduction of microbial flora found by FAVILLI *et al.* (1981) in treated plots at Cadriano had no effect on the density of the microarthropod populations.

The nil effect of atrazine was already found for other animal groups, such as Lumbricidae (SCHWERDTLE 1969) and Nematoda (FREY 1976; POPOVICI *et al.* 1977).

At Vitulazio the effects of atrazine treatment differed from those found in Cadriano and those described by POPOVICI *et al.* (1977) for microarthropod populations in a field of Oradea (Rumania). One month after treatment Acarina populations in treated plots in the Vitulazio field were statistically the same as in the control plot, whereas Acarina populations in the field treated by POPOVICI *et al.* with 5 kg ha⁻¹ (a dose similar to that used in our experiments) were reduced by 68% in comparison to control. Collembola were decreased to a lesser extent in Vitulazio. One month after treatment, the Isotomidae populations at Vitulazio were not statistically different between treated and control plots; in POPOVICI's field they were decreased by 88%. According to POPOVICI *et al.* (1977), Hypogastruridae were completely eliminated by the application of 5 kg ha⁻¹ of atrazine; at Vitulazio with dose of 6 kg ha⁻¹ Hypogastruridae were only decreased ($P > 0.05$). Comparison of the data was complicated by the fact that the Rumanian authors did not provide statistical evaluation of their percentage values and that manual weed control was not done on the control plot, as at both Vitulazio and Cadriano. The Rumanian authors realized that the presence of weeds in the control plot may have determined the differences in number of soil fauna.

The presence of additional vegetation in fields not treated with atrazine has been invoked as motive to explain differences in soil animal numbers between control and treated fields (FOX 1964). In this regard, EDWARDS & STAFFORD (1979), using both weedy and weed-free control plots evidenced that microarthropod population densities were enhanced in plots having more weeds. Since, in our experiment, the control was weed-free and the samples were taken a few days after atrazine treatment, we can state that the effects found at Vitulazio were direct.

A direct effect of other triazines (cyanazine and simazine) on microarthropod populations has been pointed out by EDWARDS & STAFFORD (1979).

The lethal and repellent effects of atrazine can be discriminated by analyzing the soil samples immediately under those affected by atrazine. The repellent effect was recognizable as an increase in population densities of the lower samples correlated to a decrease in those of upper samples.

Such analysis of the data from Vitulazio supported the view that repellent activity was uncommon: only Hypogastruridae showed this trend. The only other populations from the 10–20 cm. layer to increase significantly were those of Onychiuridae sampled in May from the 2 kg ha⁻¹ treated plot, even though the decrease in animals from the 0–10 cm layer was not significant. In other cases, large variations in the 10–20 cm layer populations were not obvious even after significant decrease in the 0–10 cm layer populations.

The overall results of our research on the effects of atrazine on experimental fields led us to the conclusion that atrazine had only very limited or nil effect on soil microarthropod populations at recommended agricultural dose of 2 kg ha⁻¹.

The different results obtained between the experimental fields at Cadriano and Vitulazio may be explained by the possibly greater binding properties of one soil in respect of the other (TAFURI 1981). Thus, the effects of atrazine could be in some way blocked by the Cadriano soil so as to lessen its action on the microarthropod populations. On the contrary, at Vitulazio, atrazine would remain free longer and have sufficient time to act on susceptible taxa.

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Synopsis: *Original scientific paper*

FRATELLO, B., R. BERTOLANI, M. A. SABATINI, L. MOLA & M. A. RASSU, 1985. Effects of atrazine on soil microarthropods in experimental maize fields. *Pedobiologia* **28**, 161—168.

Atrazine treatment of experimental fields cultivated with maize may or may not cause significant decreases in microarthropod populations living in the 0—10 cm layer. Immediate negative effects on Acarina and Collembola population density were found in one field and no effects in another. In the first field atrazine had only very limited or nil effects on soil microarthropod populations at the recommended agricultural dose of 2 kg ha⁻¹. Even in the 6 kg ha⁻¹ treated plot, any negative effects were followed by a recovery one month from treatment. The microarthropods living below 10 cm depth never showed any significant reduction in numbers.

Key words: soil, microarthropods, herbicides, atrazine, Collembola, Acarina.